(d) preparing a subsequent cell culture by combining said multipotent neural stem cell progeny with fresh substantially serum-free culture medium containing at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation to proliferate said daughter multipotent neural stem cells to produce more progeny which include more daughter multipotent neural stem cells.--

E4 cont

--~~103~~ 121. The method of claim ~~101~~ 119 wherein said culture medium is defined.--

--~~104~~ 122. The method of claim ~~101~~ 119 wherein said growth factor is selected from the group consisting of epidermal growth factor, amphiregulin, fibroblast growth factor and transforming growth factor alpha. --

REMARKS

Claim Amendments

Claim 17 is amended to include the feature of canceled claim 95 and a feature that has been deleted from claim 87. As was discussed during the Examiner's interview, the applicants have found that multipotent neural stem cells proliferate rapidly in the presence of a growth factor in a serum-free culture medium. Accordingly, claim 17 has been amended to include this feature (which has been deleted from claim 87). Claim 17 has also been amended to recite that the multipotent neural stem cell proliferated using the method is capable of producing progeny that are capable of differentiating into astrocytes. This further defines the source of the neural tissue used in the method, as discussed in more detail below. Additional minor amendments have been made to render the claim more clear.

New claims 96-100 are directed to the aspect of the invention wherein juvenile or adult mammalian neural tissue is used (as opposed to embryonic or neonatal tissue). The Examiner indicated that a claim having the feature of obtaining the cells that are cultured from adult mammalian neural tissue would overcome the art rejections (Paper No. 23, p 3, ¶ 2; p. 5, ¶ 1).

New claims 101-104 are directed to the aspect of the invention where human neural tissue is used as the source of the cells.

Rejection Under § 103

The Examiner has maintained the rejection of claims 17, 18, 20 and 85-95 as being unpatentable over Anchan taken with Boss. The Examiner relies on the Anchan reference for teaching that neural cells can be induced to proliferate in culture medium containing epidermal growth factor. The Examiner relies on the Boss reference for teaching that proliferated neural cells can be subcultured (see Paper No. 20, pages 11-14). Applicants maintain that neither Anchan nor Boss concern methods for proliferating multipotent neural stem cells, and that this feature of claim 17 would not have been obvious or suggested by these references.

Claim 17 has been further amended to recite that the multipotent neural stem cell is capable of producing progeny that are capable of differentiating into neurons and glia, including astrocytes. Anchan concerns the culture of neuroepithelial cells of embryonic and early postnatal rat retina. Such neural tissue would not be expected to contain cells capable of producing progeny that differentiate into astrocytes, and Anchan did not report the presence of astrocytes. Thus, at the time of Applicant's invention, there would be no insight gained from the Anchan reference on how to achieve a method of proliferating multipotent neural stem cells capable of producing progeny that are capable of differentiating into neurons and glia, including astrocytes.

While it is believed that the above remarks are sufficient to overcome the Examiner's rejection, Applicants further address the distinctions between the method of Boss and the method of claim 17 in view of the Examiner's reliance on this reference in rejecting the claims of related application U.S. Ser. No. 08/376,062. Boss teaches that committed neuronal progenitor cells proliferate in a culture medium that does not contain growth factors (see page 14, lines 24-29 for description of proliferation medium). Thus, it does not suggest the use of growth factors to induce proliferation of neural cells. Boss further discloses an initial culture medium supplemented with serum, which selects for neuronal progenitor cells (p. 11, lines 29-37). Thus Boss does not suggest a method of culturing neural cells where the cells have "not been treated with serum *in vitro*", as required by claim 17.

In summary the method disclosed in the Boss reference is very different than the method of claim 17. The Boss reference, taken by itself or in combination with the other references cited by the Examiner, does not teach that a multipotent neural stem cell capable of producing progeny that are capable of differentiating into neurons and glia, including astrocytes, can be proliferated *in vitro* in a substantially serum-free culture medium.

CONCLUSIONS

For the foregoing reasons, it is believed that the claims are patentable and should be allowed.

Respectfully submitted,

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